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A 'Marginal' tale: the development of the neocortical layer 1

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The development of neocortical layer 1 is a very dynamic process and the scene of multiple transient events, with Cajal–Retzius cell death being one of the most characteristic ones. Layer 1 is also the route of migration for a substantial number of GABAergic interneurons during embryogenesis and where some of which will ultimately remain in the adult. The two cell types, together with a diverse set of incoming axons and dendrites, create an early circuit that will dramatically change in structure and function in the adult cortex to give prominence to inhibition. Through the engagement of a diverse set of GABAergic inhibitory cells by *bottom-up* and *top-down* inputs, adult layer 1 becomes a powerful computational platform for the neocortex.

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Introduction

The ability to comprehend the world around us comes through a continuous integration between the external and internal representations of our environment. Our brain executes this task by handling two main streams of information: *bottom-up*, which arrives via inputs originating in our sensory organs, and *top-down*, which is initiated within the brain and regulates the incoming signals adding complex contextual content. One of the main sites where these two types of information converge and are integrated is the superficial layers of the neocortex [1••].

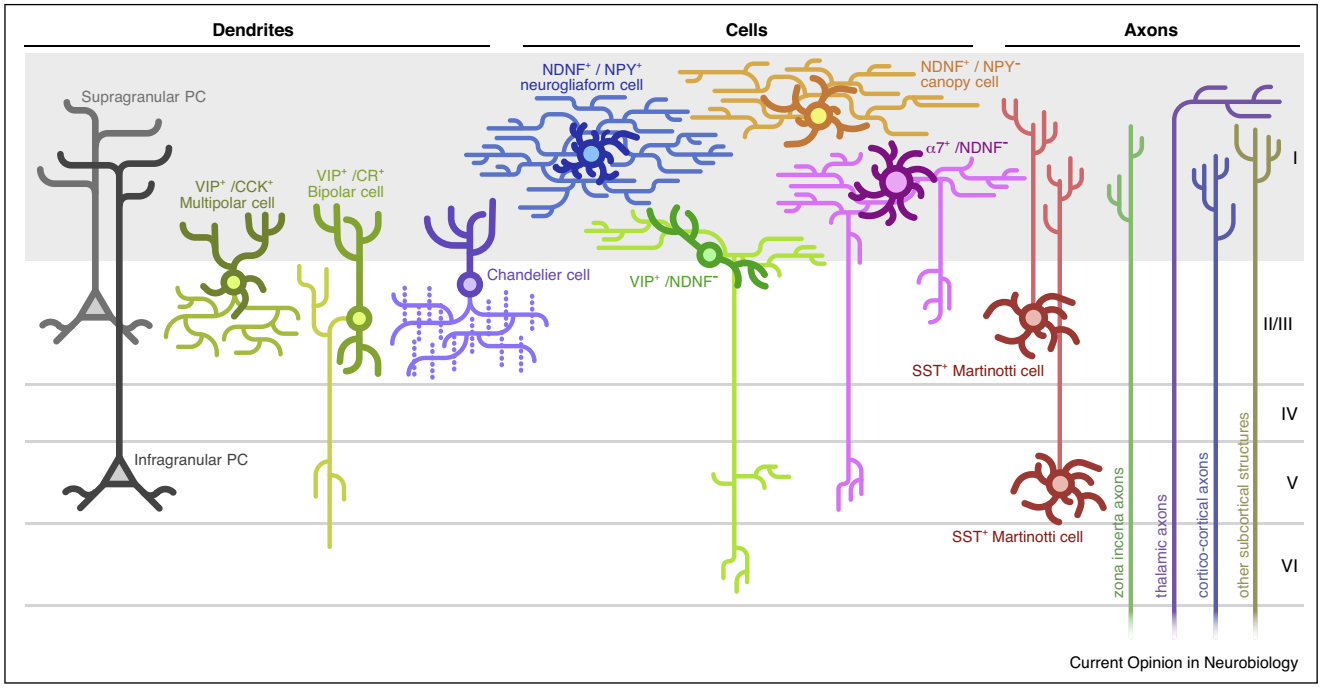
Out of the three superficial layers, adult neocortical layer 1 (L1) has an array of unique features, being occupied by only GABAergic and no Glutamatergic cells, as well as by a plethora of axonal fiber terminals and dendrites from diverse neuronal sources [2••]. The first noteworthy description of the cellular anatomy of this layer goes back to Ramon y Cajal [3]. In over a hundred years our knowledge about the morphology and activity of the most marginal layer of the brain has dramatically increased, suggesting that this is certainly not a *marginal* player in brain function. In fact, in 1998 Marín-Padilla pointed out that '*the terms 'marginal zone' and 'molecular layer' that are used frequently in reference to layer I and denote absence of neurons and barren structure, are erroneous from developmental, structural and functional points of view and should be abandoned*' [4]. Most interestingly, it has been discovered that L1 has an essential contribution throughout brain development, as it instructs the lamination of the mammalian neocortex and participates in early circuit formation. In this review, we will focus on the neuronal composition and development of L1, but, due to space restrictions, we will not cover the important role that other abundant L1 cell types may play, such as astrocytes [5]. We will begin with a description of adult L1, subsequently follow the story of its development, guiding the reader through its structural changes from embryogenesis to adulthood and finish with presenting how L1 participates in the function of the adult brain.

Prologue: the composition of adult layer 1

Adult L1 has an intriguing composition compared to the other cortical layers. It contains few somata of GABAergic inhibitory interneurons (INs) [2••], the majority of which expresses Reelin [6], and a diverse array of neuronal fibers which create a thick and intricate web (Figure 1). It is the site where many axonal projections from a variety of sources converge, including the cortex [7,8], the subthalamic zona incerta [9], the thalamus [10] and other subcortical neuromodulatory nuclei: cholinergic, serotonergic and noradrenergic fibers from the basal forebrain, the raphe nucleus and the locus coeruleus, respectively [11–14]. L1 is also the preferential target of Martinotti cell axons, a group of cortical INs that express Somatostatin (SST) as a distinguishing marker and reside in deeper layers [6]. This big assortment of axonal inputs target residing INs and the L1 dendritic tuft of both supragranular and infragranular pyramidal cells (PCs) [6]. Finally, few other types of INs located in layer 2/3, including Chandelier cells [15] and VIP^+ (Vasoactive Intestinal Peptide) multipolar and bipolar INs [16,17], extend their dendrites into L1.

Past efforts were able to identify different types of L1 INs using blind *in vitro* electrophysiological recordings and

Figure 1



The structure of adult layer 1.
This schematic depicts the composition of adult neocortical L1, which contains a few GABAergic cells and a number of neuronal fibers, which create a thick and complex network. *Dendrites:* several cells have their apical dendrites branching within L1, including layer 2/3 and 5 pyramidal cells and multiple layer 2/3 GABAergic interneurons (VIP$^+$/CCK$^+$ Multipolar cells, VIP$^+$/CR$^+$ Bipolar cell, Chandelier cells). *Cells:* In accordance with Schuman *et al.* [2••], there are at least four molecularly, morphologically and electrophysiologically distinct GABAergic cell types present in L1. NDNF$^+$/NPY$^+$ neurogliaform cells have highly elaborate axons that ramify in L1, are late spiking and have slow synaptic output. NDNF$^+$/NPY$^-$ canopy cells tend to sit closer to the pia surface and have a similarly elaborate axon that can give off collaterals that project into deeper layers (not shown). They fire early upon depolarization and also have a slow synaptic output, without activating postsynaptic $GABA_B$ receptors. The other two types, the α7 nAChRs$^+$ cells and the VIP$^+$ cells are relatively closer to the border of L1 and 2 and have axons that ramify in L1, with several collaterals into deeper layers. The former discharges action potentials in a more bursty manner with a large after-depolarization potential (ADP), whereas the latter shows a more irregular firing pattern. *Axons:* L1 receives both non L1 intra-cortical and extra-cortical axonal projections. The former include GABAergic inputs from Martinotti cells located in layer 2/3 and layer 5, but also callosal fibers from contralateral supragranular pyramidal cells. The latter, comprise of zona incerta GABAergic axons, thalamocortical glutamatergic axons from Calbindin$^+$ neurons located in several thalamic nuclei (sensory, motor and higher order), cholinergic fibers from basal forebrain, serotonergic inputs from the raphe and noradrenergic axons from the locus coeruleus. (Each cell type and long-range process is color-coded. The cell bodies are round filled with bright color; the dendrites of the cells are of the same color as the outline of the cell body and are depicted in thick lines, whereas the axons in thinner lines of lighter color; the characteristic cartridges of axonal terminals of chandelier cells are shown as dots).

anatomical reconstruction of their axo-dendritic neurites [18,19••,20,21]. In the past few years, the advent of single cell RNA sequencing allowed for a better distinction of multiple neuronal types from the molecular point of view and hence also the generation of transgenic animal lines to label and fate-map subpopulations of INs [22,23••,24•,25•,26•]. Taking advantage of these developments, a recent study expanded on the cellular composition of L1 and found four unique IN types which are molecularly, morphologically and electrophysiologically distinct: NDNF$^+$/NPY$^+$ neurogliaform cells, NDNF$^+$/NPY$^-$ canopy cells, α7 nAChRs$^+$/NDNF$^-$ cells, and some multipolar VIP$^+$ cells [2••,27] (Figure 1). A comprehensive account of the most recently published work that has addressed L1 IN diversity is presented in Box 1.

Even though a unique aspect of adult L1 is being devoid of glutamatergic cells, intriguingly this is not the case during development, where a series of transient events make the plot of this story more intricate.

Act I: a story of tangential migration

At the beginning of embryogenesis, the neocortex is composed of two adjoining sheets: the Ventricular Zone (VZ), which contains neural stem cells, and the Preplate (PP), which lies over the VZ and is populated by the

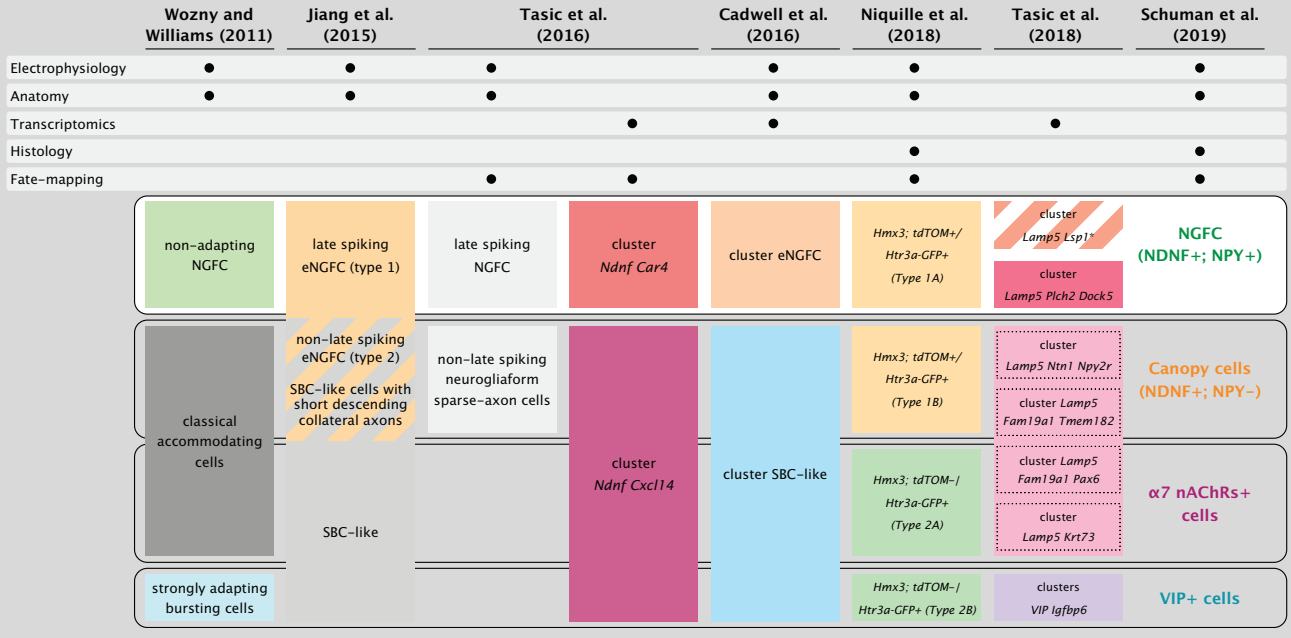
Box 1 GABAergic interneuron types in adult layer 1

In the recent study of Schuman *et al.* [2••] the authors reported four distinct types of L1 INs after a comprehensive morphological and electrophysiological characterization. Moreover, they identified a handful of unique molecular markers that help in the distinction of each type (see also Figure 1). However, in the past few years, several laboratories have explored L1 composition using a combination of techniques. In this box, we make an effort to map previously described L1 IN diversity onto the four cardinal types described in Schuman *et al.*

All studies agree on the distinction between neurogliaform cells (NGFCs) and a heterogenous population of single bouquet cells (SBCs), with the first being late spiking with axons that ramify in L1 and the second being non–late spiking with a heterogeneous morphology and several collateral axons in deeper layers. Schuman *et al.* argue that the classical accommodating cells described in Wozny and Williams study [20], likely correspond to both canopy and α7 nAChRs$^+$, whereas the strongly adapting bursting cells match their VIP$^+$ cells. Niquille *et al.* showed that NGFCs originate from a pool of *Htr3a*$^+$/*Hmx3*$^+$ cells in the preoptic area. Within the NGFC population, they distinguished between two electrophysiological cell types, 1A and 1B. Close observation of the recorded properties suggests that type 1A correspond to NGFCs, whereas 1B to canopy cells. At the same time, it could be claimed that within the *Htr3a*$^+$/*Hmx3*$^-$ (SBC-like cells) type 2A matches the α7 nAChRs$^+$ group, characterized by a big ADP after spiking, and type 2B to the VIP$^+$ cells group, with a less prominent ADP.

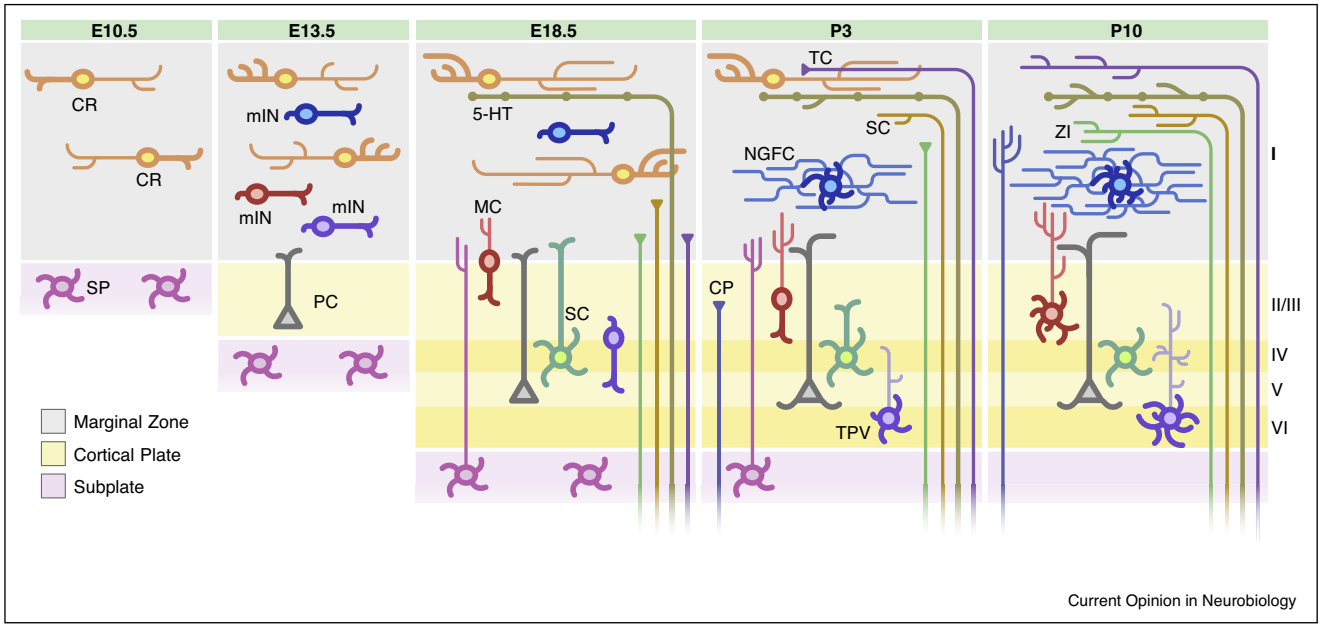
In line with morphological and electrophysiological differences, NGFC and SBC-like cells are also transcriptionally distinct as confirmed by single cell RNA sequencing from Tasic *et al.* [23••] and patch-RNA sequencing from Cadwell *et al.* [22]. In 2018, Tasic *et al.* published a more detailed transcriptomic parcellation of L1 INs, identifying several subtypes including the cluster *Lamp5/Plch2/Dock5*, which corresponds to typical NGFCs, and four additional *Lamp5* clusters that, most probably, span canopy and α7 nAChRs$^+$ cells. It is noteworthy that a closer examination of cluster *Ndnf/Cxcl14* (Tasic *et al.* [23••]) revealed many *Ndnf*-negative cells, suggesting that this might be a heterogenous cluster that could include both canopy and α7 nAChRs$^+$ cells. Moreover, clusters *VIP/Igfbp6* from Tasic *et al.* [25•] map almost completely onto cluster *Ndnf/Cxcl14* of the previous dataset, suggesting that also some VIP$^+$ cells may have been included in this cluster. Similarly, cluster *Lamp5/Fam19a1/Pax6* and cluster *Lamp/Krt73* (Tasic *et al.* [25•]) are largely *Ndnf*-negative, suggesting that, among SBC-like cells, the majority of *Ndnf*-expressing cells are, in fact, canopy cells. Finally, based on their mRNA expression of *α7 nAChRs*, it is not unlikely that these two clusters are mainly α7 nAChRs$^+$ cells.

(* Only a small fraction of cluster *Lamp5/Lsp1*, and therefore, a small number of cells, maps onto cluster *Ndnf/Car4* from Tasic *et al.* [23••]. Box colors and cell definitions are the same found in the original papers.)



first post-mitotic differentiated neurons: the glutamatergic subplate cells (SPcs) and Cajal–Retzius cells (CRcs) [28]. The latter have been shown to be born from embryonic day (E) 9.5 to E11 at distinct focal sources (the cortical hem, the septum and the pallial-subpallial boundary (PSB) [29••,30••,31]) and migrate tangentially forming a uniform layer at the very top of the cortex [32,33••,34], the diversity of which is under investigation [35]. Starting from E12.5, several waves of newborn PCs migrate into the PP generating what is called the Cortical Plate (CP) which splits the PP in two layers: a lower one named 'Subplate' (SP), occupied by the SPcs, and an upper one called 'Marginal Zone' (MZ), populated by CRcs [28]. Recently, a novel source of cortical CRcs was identified [36]. In fact, despite the massive expansion of the neocortex, the density of CRcs

Figure 2



A time-course of marginal zone development.
This schematic depicts how the MZ gets populated by different neurons and neuronal elements over development. CRcs are the first neurons to tangentially migrate into the MZ around E10.5, tiling the cortical surface by E12.5. Even though their neurites elaborate over the next few days, their number decreases drastically after birth and they disappear almost completely by P10. GABAergic cortical INs – the second migratory wave – begin migrating through the MZ around E12.5. Some IN types preferentially migrate through the MZ, such as SST$^{\pm}$ Martinotti cells and Translaminar Parvalbumin^{+} (PV) cells. By E18.5, GABAergic cells migrating through the MZ start moving radially into the appropriate layer of the developing neocortex, where they start acquiring their typical morphology. SST$^{\pm}$ Martinotti cells dive into the cortex leaving their nascent axon in L1. Infragranular and supragranular pyramidal cells have apical dendritic tufts branching within the MZ already as they are migrating into the developing CP. Contralateral callosal projections from supragranular cells arrive in L1 during the first postnatal week, as are Zona Incerta GABAergic projections. Layer 4 stellate cells project an apical dendrite to L1, which is retracted during the first postnatal week. At the end of embryogenesis, L1 receives first serotonergic projections (5-HT) and then thalamocortical, noradrenergic and cholinergic innervation: all these projections expand drastically during the first postnatal weeks. (Each cell type and long-range process is color-coded. The cell bodies are round filled with bright color; the dendrites of the cells are depicted in thick lines, whereas the axons in thinner lines of lighter color; CR, Cajal–Retzius cell; mIN, migrating interneuron; PC, pyramidal cell; NGFC, GABAergic neurogliaform cell; TPV, GABAergic translaminar Parvalbumin^{+} cells; MC, SST$^{\pm}$ GABAergic Martinotti cell; SC, stellate cell; TC, thalamocortical axon; 5-HT, serotonergic projections; SC, subcortical projections; CP, callosal projections; SP, subplate cell projections; ZI, zona incerta projections).

keeps increasing between E14.5 and E15.5. Interestingly, this appears to be not because of the generation of new CRcs, but rather because of a recycling process of differentiated olfactory CRcs, which initiate a second wave of migration from the lateral olfactory tract to the MZ [36].

CRc tangential migration is a complicated and only partially understood process. In the last couples of decades, multiple factors have been shown to be involved in controlling the proper positioning of CRcs, including external signaling cues, cell-autonomous transcriptional programs, cell-to-cell repulsive interactions and finally the presence of a physical substrate. A detailed account of all these mechanisms lies beyond the scope of this review; for a comprehensive overview, we refer the reader to Barber and Pierani [37] and [38]. Beyond the mechanisms of how CRcs reach their final position, their presence in L1 contributes to multiple aspects of early brain development, including cortical arealization [39,40] and the expansion of superficially located neurites. Evidence comes from experimentally decreasing the density of CRcs, which leads to the partial invasion of layer 2/3 PC bodies in the MZ, but fewer axons and dendrites extending into it. In contrast, an increase of CRcs stimulates the development of superficial dendritic tufts and axonal arborization, increasing the thickness of the MZ [36].

Around E12.5, the MZ is the scene of a second migratory stream (Figure 2). Cortical INs are generated from discrete proliferative regions within the subpallium during the second half of embryonic development, the ganglionic eminences and the preoptic area [26•,41]. After becoming

post-mitotic, INs migrate tangentially into the developing neocortex either through the subventricular zone (SVZ) or along the MZ. Around E18.5, INs leave the migratory streams and invade the CP finding their place in the destined layer. The distribution of INs within the two migratory streams is not equal and only 25% of them migrate through the MZ [42•]. Interestingly, INs of the two streams have differential transcriptional profiles [43•], indicating that they are not randomly sorted between the two routes. In fact, it was recently shown that the INs destined to become the $SST^{\pm}$ Martinotti type need to migrate preferentially through the MZ stream in order to properly develop, and that the same route is also taken by the majority of Translaminar $Parvalbumin^{+}$ (PV) cells [42•]. In line with the path being occupied by CRcs, IN positioning has also been observed to depend on the former cells. Specifically, it has been shown that the loss of CRcs or their abnormal distribution affects INs migration in the MZ [44–46] and the final allocation of L1 $PROX1^{+}$ INs [36]. Several studies have identified a number of key cues that control the migration of developing INs through the MZ (for an extensive description we point the reader to Guo and Anton [47]).

As both CRcs and some INs share the MZ for their cortical migration, it is of no surprise that both cell populations respond to similar cues guiding their movement and determining their final position. Some of these cues are guidance molecules provided by the neighboring meninges, which also act as a physical substrate for migrating cells. Specifically, the meninges release the chemokine CXCL12 that attracts CRcs to the pial surface through the interaction with the canonical receptor CXCR4 (shown to be expressed by hem-derived CRcs) and the alternative receptor CXCR7 (expressed by both hem-derived and PSB-derived CRcs) [48–50]. Similarly, the expression of CXCR4 in INs prevents them from invading the CP ahead of time [51,52] and it is believed that this may occur through the action of CXCL12 on the branching of the leading process of migrating INs. When CXCL12 is reduced, the branching is enhanced and migration rate becomes slower, increasing the probability of exiting the migratory streams towards the CP [53].

Entr'acte: REELIN, radial migration and the evolution of the mammalian neocortex

The cerebral cortex is an evolutionary homology common to all amniotes [54]. However, the mammalian neocortex displays a unique highly organized six-layered structure. Notwithstanding the plethora of different roles reported for CRcs [36,39,40,55], the regulation of cortical layering through the release of the glycoprotein Reelin is the one they are best known for [32,33••,34]. Several anatomical comparisons between mammals and other amniotes have proposed that the release of Reelin at the MZ is an important step in the evolution of the mammalian cortex. Reelin-expressing cells can be found in all amniotes, in particular at the surface of the dorsal pallium. However, they are fewer in number and weaker in Reelin expression in reptiles and birds (Figure 3a) [56]. In line with this, the non-avian reptilian brain has a simpler single-layered structure, while birds lack any lamination, having instead a nuclear organization [56]. One thesis posits that the huge accumulation of CRcs, and therefore, the high levels of Reelin released from the MZ may have contributed to the complex lamination of mammals [56]. In support of this argument, it has been shown that ectopic overexpression of Reelin in the pallium of birds increases the extension of radial glia scaffold and changes the morphology of migrating newborn neurons into a bipolar shape, more similar to the ones present in mammals [57].

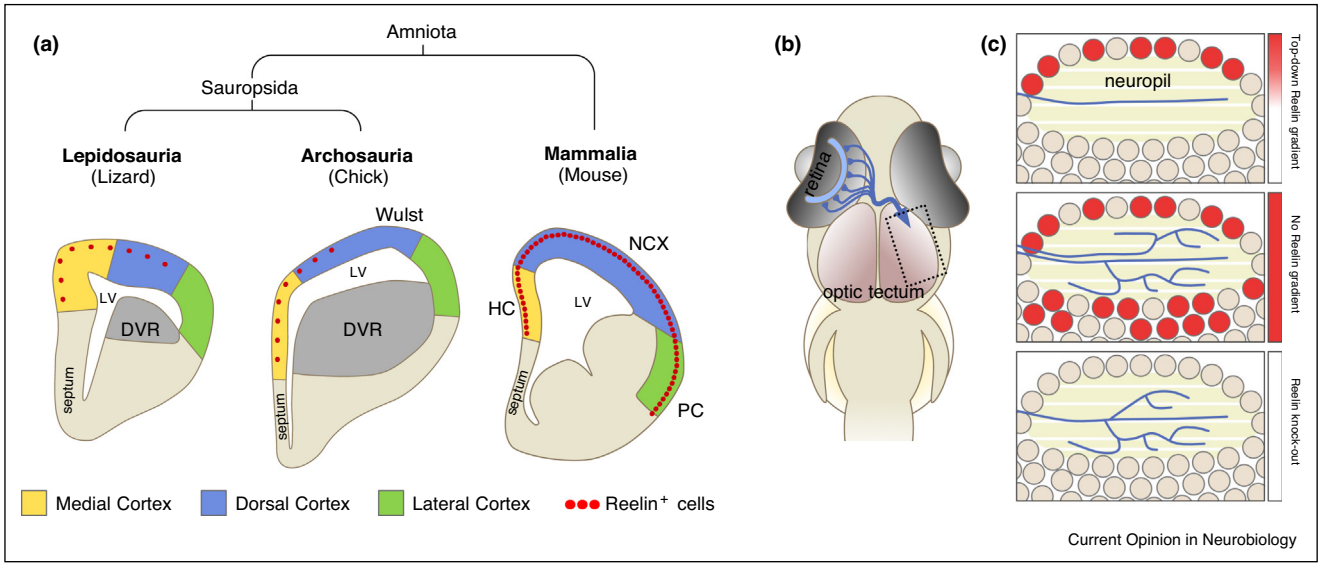
Nevertheless, Reelin displays an important guidance role also outside the amniotes group [58••,59]. For example, in Zebrafish a population of inhibitory INs located in the most superficial layers of the optic tectum expresses Reelin in a dorso-ventral gradient, which is important for the lamination of retinal ganglion axons into the tectal neuropil (Figure 3b and c) [58••]. Interestingly, Reelin is also expressed by a subset of mammalian neocortical INs many of which sit in L1. In contrast to that in CRcs, the function of Reelin in these cells has not been revealed, although it has been postulated that Reelin can play an important role in synaptic plasticity later on in life [60].

Act II: the development of the net

Besides CRcs, INs and their processes, the MZ is populated by externally originating dendrites and axons, the development of which is not fully worked out (Figure 2).

As CRcs tile in the neocortex, they extend a long, yet poorly ramified axon along the pia mater and a main apical dendrite originating on the opposite side of the cell body [61]. Most probably, CRcs receive the first synaptic contacts on their dendrites by the axons of their PP partner SPcs [62], another largely transient population of neurons that sits in future layer 6 and contributes to cortical construction [63,64]. During CP formation, migrating PCs already have branching apical dendritic tufts within the MZ. In fact, the leading process, which guides neuronal migration, directly transforms into the apical dendrite and starts ramifying upon contact with the MZ. This is linked to the end of cell movement since the soma stops below the first stable branch point of the nascent dendrite [65]. Rabies-based tracing in the first postnatal week revealed that the apical dendrites of PCs receive transient connections from CRcs [66•], which are themselves contacted by layer 5 $CTIP2^{+}$ PCs [66•], thereby creating a cortical connectivity loop. At the same time, supragranular PCs start sending their axons to the contralateral L1, which arrive between P5 and P10 through a conspicuous amount of callosal fibers [7,8]. Interestingly, the developing MZ is also transiently targeted by dendrites of the layer 4 spiny stellate cells, which are nevertheless

Figure 3



Reelin and the evolution of cortical lamination.
(a) All amniota have Reelin-expressing cells during brain development; however, mammals display a higher number which tiles the whole neocortical surface (in the cartoon, a schematic of a coronal section of the mouse brain at E15.5 and the developmentally comparable coronal sections of the lizard and the chick brain are shown). Red dots represent Reelin-expressing cells: CRcs in mouse and CR-like cells in lizard and chick. **(b)** Zebrafish retina projection cells send axonal inputs (blue lines) to recipient layers located in the optic tectum. **(c)** Enlarged dotted box shown in '(b)'. The precise lamination of retinal axonal inputs to the optic tectum neuropil depends on Reelin. Reelin is expressed in a top-down gradient within the neuropil. Disruption of this gradient either by introducing an extra source of Reelin or by complete removal alters the proper retino-axonal lamination. Red full circles represent Reelin-expressing cells. (LV, lateral ventricle; DVR, dorsal ventricular ridge; HC, hippocampus; NCX, neocortex; PC, piriform cortex).

retracted between P3 and P5 [67•,68•]. In contrast, the length of apical dendrites of supragranular and infragranular PCs increases significantly between P7 and P14 [69,70].

Besides intracortical connections, L1 is also the target of several subcortical projections. The earliest ones to arrive are serotoninergic fibers from the dorsal and the medial raphe nuclei, which invade the developing cortex by E16 [12] and have been reported to play an important role in L1 development. In fact, the removal of the serotonin receptor 5-HT3$_A$ from caudal ganglionic eminence-derived Reelin$^+$ INs leads to their mis-positioning [71]. Moreover, the same receptor type was found to induce Reelin release from CRcs that, in turn, mediates pruning of the PC apical dendrites in the MZ [12].

Around the same time as serotonergic fibers reach the developing cortex, L1 also starts being targeted by the axons of Calbindin$^+$ neurons located in several thalamic nuclei. Some of these axons originate from the so called 'nonspecific' thalamocortical projection neurons and display a flat and extensive subpial arbor which is exclusive to L1; others given out by the 'multispecific' thalamocortical projection neurons also have branches in other cortical layers and axonal collaterals in subcortical regions. In rodents, the growth cones of both types of axons extend through the CP all the way to the MZ, with virtually no branching in other layers. Once they reach the upper border of the CP at the end of embryogenesis, they pause for around 48 hours before taking a 90° turn to start extending tangentially within the L1a [10]. Importantly, some of these thalamic axons carry sensory signals that are essential for the development of the morphology and connectivity of L1 Reelin$^+$ INs [72••,73••]. Even though there are data suggesting that many of the inputs onto these INs are lost in the somatosensory cortex during the second postnatal week [74••], adult L1 INs of the prefrontal cortex have been found to receive thalamic inputs, which are able to drive their activation *in vitro* and hence the generation of feed-forward inhibition onto PC dendrites [75].

Later in development, the locus coeruleus and the basal forebrain send noradrenergic and cholinergic axons respectively to the cortex, which branch extensively in L1 between the first and the second postnatal week [13,14]. In addition, it was recently reported that subthalamic zona incerta GABAergic neurons project heavily to L1 from at least the first postnatal week onwards and

that their early activity regulates synapse formation onto L5 PCs [9].

Finally, also during the first postnatal week, resident L1 INs develop extensively ramifying axons, either exclusively within L1, or with few collateral branches towards the infragranular layers (Figure 1). In fact, NDNF$^+$ L1 INs have some of the most intricate and dense axonal arborizations of any neuron in the whole brain [2••,76•,77••]. Nevertheless the developmental mechanism governing their elaborate axonal projection pattern is almost completely unknown [73••]. In contrast, their dendrites are not as complex and receive cholinergic inputs from layers 2/3 ChAT$^+$ VIP$^+$ bipolar cortical cells [78], the developmental role of which is yet to be explored. Intriguingly, some of these L1 Reelin-positive INs (not examined for NDNF expression) [66•], as well as other INs from deeper layers, some of which express SST, Calbindin or Reelin, have been shown to connect to hem-derived CRcs in the first two weeks of postnatal life. These anatomical findings are in line with previous research that has reported two distinct GABAergic functional inputs of unknown origin onto CRcs [79] and together demonstrate that CRcs participate in functional circuits before their demise.

Epilogue: the dusk of Cajal Retzius cells

In their final act, CRcs disappear from the neocortex through extensive cell death during the first two postnatal weeks. Distinct subtypes of CRcs show different dynamics of death; most of PSB-derived CRcs die early, between P1 and P4, whereas septum-derived and hem-derived CRcs (the majority of cortical CRcs) show a sharp drop at P10. Notably, septum-derived CRc death depends on the pre-apoptotic factor BAX, whereas hem-derived CRc death is triggered by a different unknown pathway [80•]. By the third postnatal week very few CRcs are left in L1 of the neocortex, even though and intriguingly a minor fraction of hem-derived CRcs survives in adult visual and anterior lateral motor cortex [25•], as well as in the hippocampus [81].

Interestingly, GABA-related activity has been proposed to participate in CRc demise; in fact, CRcs never upregulate the transporter KCC2, nor downregulate the transporter NKCC1, with both events leading to a high intracellular concentration of Cl^- and therefore, a depolarizing effect of GABA. Pharmacological inhibition of GABA signaling *in vitro* and inactivation of NKCC1 *in vivo* are sufficient to rescue some CRcs from death, suggesting that GABA, released by connected INs, may induce an excitotoxic effect on postnatal CRcs [82•,83].

The fact that CRcs are active components of early circuits opens an important question about their death, not only in terms of which presynaptic INs may drive it, but also whether it serves a key role for the final setup of cortical networks. One way to assess this is to prevent CRc death. This has been performed for septum-derived CRcs, either by knocking out *Bax*, which prevents apoptotic cell death, or by overexpressing the potassium channel Kir2.1, which hyperpolarizes the cells, hence probably preventing them from death by excitotoxicity. Survived CRcs are morphologically similar to early postnatal CRcs, have electrophysiological properties of immature neurons and still receive GABAergic inputs. It has also been shown that the survival of CRcs through removal of *Bax* triggers the development of exuberant dendrites and spines of layer 2/3 PCs, and consequently an increase of excitatory synaptic inputs [55,80•]. Taken together, these results suggest that CRc death does not represent a mere step of cell removal, but instead an essential mechanism required for shaping cortical networks, which merits further investigation.

Closing applause: the rise of inhibition

At the end of our story, few, but diverse inhibitory cell types are still standing on the proscenium of the brain. What was a stage of largely excitation during development has become an umbrella of inhibition over the adult neocortex. Having a superficial layer of highly arborized inhibitory cells can offer a great advantage from a computational point of view. The wide variety of sensory-driven information that reaches L1 can be selectively gated by cortico-cortical and neuromodulatory inputs that differentially engage highly specialized GABAergic cells residing or having their dendrites in this layer. In the adult, for example, it has been shown that L1 NDNF$^+$ INs display increased inhibition of the apical dendrites of layer 2/3 PCs during behavioral learning [1••], as well as that unidentified L1 inhibitory cells are key in gating cross-modal sensory integration [84••]. In addition, interhemispheric communication impacts the integration of excitation onto the L1 portion of L5 PC dendrites through the interplay between NMDA and slow $GABA_A$ and $GABA_B$ receptor activation [85]. The latter is most likely provided by NDNF$^+$ INs, the impact of which may also be altered through the extensive presence of electrical synapses between them, by synchronizing or desynchronizing their action potential discharge [86,87].

Despite the handful of reports about the involvement of L1 cells in the function of the adult cortex, there remains much to be discovered in order to start comprehending their role in sensory coding and behavioral performance. Given this, it is no surprise that we know even less about the contribution of L1 resident cells and invading neurites in the developmental function of the cortex [74••,88•]. An intriguing phenomenon that may prove to be key in unveiling the function of the developing L1 in the structural and functional assembly of the neocortex, is the 'change of guard' between CRcs and INs, and hence a shift from overall excitation to

inhibition, mostly during the second postnatal week. Fascinatingly, it is in fact also at the end of the second postnatal week that mice undergo a behavioral transition from a 'passive' to an 'active' state, with all sensory modalities transmitting signals and a sharp increase in animal locomotor activity [89]. To fully comprehend the developmental role of L1 one should focus not only on the huge structure-function alterations taking place in this layer, but also on the elements that remain constant, such as the key released factor Reelin, the transcription factor COUP-TFII [24•] and others (unpublished data from our lab), the continued function of which is almost completely unknown. It is without doubt that the story of the 'marginal' layer is to be continued.

Conflict of interest statement

Nothing declared.

Acknowledgements

Because of limited space, we were not able to cite all the relevant publications. Hence, on a few occasions, we cited outstanding reviews that give a comprehensive overview of some topics. We apologize to scientists whose work was not included. We would like to thank Ali Özgür Argunşah, Sara Bottes, Olivia Hanley and Rasmus Vighagen for the feedback on this manuscript. Research in the T.K. laboratory is supported by the European Research Council (679175) and the Swiss National Science Foundation (SNSF, 31003A_170037).

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